JOURNAL OF NEMATOLOGY

Official Journal of

THE SOCIETY OF NEMATOLOGISTS

VOLUME 14

Quarterly in

1982

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JOURNAL OF NEMATOLOGY

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RESEARCH NOTES

Erratum: Pages in JOURNAL OF NEMATOLOGY 14(3) should have been numbered 279-426.

Information for Contributors

Original papers on basic, applied, descriptive, or experimental nematology are considered for publication. Other categories include research notes (not longer than four pages of copy, including tables and figures); reviews developing new concepts, hypotheses, or theories; and abstracts of papers presented at the annual meetings. At least one author must be a member of the Society of Nematologists, except that nonmember staff of subscribing institutions outside the United States or Canada may submit one paper per volume.

Manuscripts: All manuscripts will be in English, although abstracts may be in a "congress" language other than English. All copy, including tables, figure legends, and literature citations, should be typed with double spacing on 8 1/2 × 11-inch manuscript paper with wide margins all around; words should not be broken (hyphenated) at the end of lines. Authors should have the manuscript critically reviewed by two colleagues whose names should be included in the letter of submission. Three copies (the original and two Xerox or similar quality copies) should be sent to the editor-in-chief with the understanding that publication is to be exclusively in the JOURNAL OF NEMATOLOGY. Papers will be reviewed by specialists in the field and edited by selected editorial staff members.

Address: Dr. J. A. Veech, Editor-in-Chief, JOURNAL OF NEMATOLOGY, National Cotton Pathology Research Laboratory, P. O. Box JF, College Station, TX 77840.

Style: The *CBE Style Manual,* Fourth Edition, and *Webster's Third New International Dictionary* will be guides for form, style, and spelling. Use SI (Systeme International) units of measurement; if British and Fahrenheit are also desired, include them in parentheses. Author(s) name(s) and page number should appear in the upper right corner of each page. The title page should be numbered 1, the abstract page 2, the first page of the text 3, etc.

Title page: Should contain the title, name(s) of author(s), a hairline over the notation "Received for publication _____"; footnote(s) giving institutional affiliation(s), senior author's address (may be the same), and acknowledgments (if any) and, finally, a condensed running head of no more than 60 spaces (including author's last name). The title should utilize strong key words and be brief, preferably not more than 100 spaces. Scientific names of plants and animals should be given. Trade names should not be used in the title.

Abstract page: Should contain an abstract—no more than 1,300 spaces—and key words *not* appearing in the title. (See Journal for style.) The abstract serves as the summary.

Body: The body of the manuscript should be organized after the introduction (do not use the word "Introduction") according to "Materials and Methods," "Results," "Conclusions," "Discussion," "Literature Cited," "Tables," "Legends for Figures," and "Figures."

Literature citations: Cite by number in parentheses in the text. Under "Literature Cited," list (double spaced, no underlining) on a separate page alphabetically by senior author and numbered consecutively. Each citation should be checked against the original publication to verify author's name, year, correct title, journal name (or publisher's name and location), volume number, and inclusive paging. Journal names will be abbreviated as in the "List of Serials" in *Biological Abstracts.*

Tables: Number (Arabic) each table and place on a separate page with descriptive title at the top. Data will appear in Arabic numerals with minimal footnotes identified by symbols as indicated in the *CBE Style Manual.* Vertical lines must be avoided. Table dimensions should consider printed page size limitations.

Figures: Number (Arabic) all "Legends for Figures" and type consecutively on a single page. In planning large illustrations, remember that a printed page is 140 mm wide × 213 mm deep. Make allowances for the legend. Combine and mount several illustrations into composite plates when possible. Trim waste from figures and leave a space of 1–2 mm between photographs in a composite. Do not send original illustrations of taxonomic papers until requested, but provide a complete set of figures (photocopies okay) with each copy of each manuscript. Lettering on illustrations should be of high contrast, neat, and at least 1 mm high after final reduction. Identify each figure on the back—*lightly* with a *soft* pencil— with "J. Nematol.," manuscript number, author's name, figure number, and width desired. Color illustrations and folded inserts can be printed only if the extra costs are paid for by the author.

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Biological nomenclature: Authors should follow the "International Rules of Nomenclature" in naming organisms; each should be identified by genus, species, authority (if applicable), varietal names (in single quotes), breeds, races, and accession numbers. Complete authorities and dates are required in taxonomic papers. Binomials should be underlined in typewritten copy (but not in "Lit. Cited" list) and fully spelled out the first time they are used, either in text, figure captions, or table titles. When new names of nematodes are introduced, the recommendations of the "International Code of Zoological Nomenclature," 1961, should be strictly applied, especially regarding type designation and statements indicating where such type material is deposited.

Chemical nomenclature: Use approved common (generic) names where available; otherwise use capitalized trade names followed by their chemical names. State the concentration of active ingredients. Chemical doses should be reported as amount of active or technical material per unit area, volume or weight (e.g., 10 ml/1,000 m^2, 10 μg/ml, or 10 mg/kg). The chemical formulation should be given and method of application clearly stated.

Editorial page change: In order to partially cover the cost of publishing manuscripts in this journal, the Society suggests a contribution of $35 per printed page, as it is impossible to meet the demand for journal space without some financial assistance. Payment is not a condition of publication, however, since articles are accepted on the basis of merit.

Separates: No free copies. Quotation and order blanks for reprints will be sent with proofs. Plates of figures are available upon request.

JOURNAL OF NEMATOLOGY

VOLUME 14 JANUARY 1982 NUMBER 1

Plant Resistance to Nematodes: Symposium Introduction[1]

DAVID T. KAPLAN[2]

Journal of Nematology 14(1):1-2. 1982.

The resistant plant is man's most energy efficient and environmentally safe means of minimizing yield losses due to plant pathogenic nematodes. Significant numbers of scientific years are being invested in research aimed at developing, understanding, and/or assessing resistant cultivars.

Traditionally, the development of nematode-resistant cultivars has included four stages.

I. Recognition and assessment of nematode damage. Such studies identify the need and justify the investment required to develop resistant varieties.

II. Genetic manipulation: sexual recombination of plant germplasm to introduce nematode as well as other types of resistance into plants with desirable agronomic or horticultural traits. Breeders and nematologists often collect wild plant species from their geographic origins for incorporation into breeding schemes.

III. Progeny evaluation: the screening of progeny to assess the influence of the nematode on plant growth and the plant's influence on nematode reproduction. Progeny which perform well are taken to the field.

IV. Field performance. An assessment is made of plant yield, growth, and nematode reproduction-population dynamics under field conditions.

This approach has been reasonably successful. Resistant cultivars are available in such crops as beans, citrus, cotton, tomatoes, and tobacco. However, attempts to develop nematode resistance in some other crops have been thwarted by an apparent lack of resistant germplasm (Cucurbitaceae infected by *Meloidogyne spp.*). In other instances, resistance is known to occur in wild species found to be sexually incompatible with their agronomically desirable relatives.

Technological advances in tissue culture techniques, somatic hybridization, and genetic engineering may be useful in solving problems of sexual incompatibility. They may also aid programs by shortening the time period involved in cultivar production (this is particularly important in perennial crops where several years growth occurs before flowering). And they may increase field resistance stability by increasing the potential for incorporating multiple genes or horizontal resistance into agronomic crops. Protoplast procedures are being examined and adapted to introduce nematode resistance into crops where none previously existed.

The value of research on the physiology of resistance has been generally overlooked in the process of developing resistant varieties. Research results on the mechanisms of plant incompatibility to nematodes have generally been treated as descriptive facts. However, definitive results in this area could provide useful tools and insights into the development of resistant varieties. Such research could, for instance, be used to guide plant breeders in the selection of parent crosses by identifying independent mechanisms of resistance and their germplasm sources. Identification of causal factors for incompatibility in nematode-plant interactions could also provide the breeder with better evaluation procedures. Incorporation of new research findings and techniques into

Received for publication 1 September 1981.

[1]Symposium introduction presented at the annual meeting of the Society of Nematologists, 16-19 August 1981, Seattle, Washington.

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cooperative breeding programs should facilitate the design of new nematode-resistant cultivars.

Finally, prior to release, the cultivar should be carefully studied to determine its level of resistance to parasitism (including its response to various types or races) and its effect on nematode reproduction. The prolonged use of resistant cultivars is dependent upon providing the grower with, not only seed, but a formula for success based on an integrated approach to nematode control.

Phytoalexins and Their Role in the Resistance of Plants to Nematodes[1]

Joseph A. Veech[2]

Abstract: Phytoalexins are antibiotic compounds synthesized in an infected plant in response to infection. Nematodes are capable of eliciting phytoalexins in resistant plants. Resistant lima bean (*Phaseolus lunatus*) infected by *Pratylenchus penetrans* produces the phytoalexin coumestrol; soybean (*Glycine max*) infected by *Meloidogyne incognita* produces glyceollin; cotton (*Gossypium hirsutum*) infected by *M. incognita* produces terpenoid aldehydes. *Key words:* review, physiology, host plant resistance.
Journal of Nematology 14(1):2-9. 1982.

In their daily existence plants may be exposed to a multitude of organisms. Most of the organisms cause no apparent harm. Organisms constituting a biological threat are often thwarted successfully. Thus, it is axiomatic that plants are resistant to most of the organisms they encounter (for to be otherwise would be disasterous to the perpetuation of the plant species). Inherent in this concept are two general types of resistance: one predicated on constitutive factors that preclude infection—preinfection resistance, and the other on factors that unfold after infection—postinfection resistance.

Preinfection resistance is probably the most common type of resistance, and more often than not the plant involved in the relationship is considered a "nonhost" of the organism(s) it encounters. In postinfection resistance the plant becomes infected but it does not succumb to the advances of the hostile organism. This type of resistance may involve constitutive morphological or biochemical factors, or it may depend on the plant's response to infection. The plant's response may involve the production of morphological barriers that sequester the infecting organism or, it may involve the synthesis of certain biochemicals that interfere with the subsequent development of the pathogen. Among plant biochemical responses to infection are the synthesis of hydrolytic enzymes, protein inhibitors, and phytoalexins. The role of phytoalexins in the resistance of plants to nematodes is the specific subject of this paper.

PERSPECTIVES

The phytoalexin theory attempts to describe a mechanism of host plant resistance to pathogens. The theory is neither new nor static. From its introduction in 1940 (20) it has been subtly modified and adjusted to accommodate new developments (3,7,11,15, 16,17,19). I interpret the theory to say that resistance in plants may be manifested in the ability of the plant to respond to infection by producing antibiotics that limit the spread or development of the invading organism.

Cruickshank (7), Bell (3), and Kuc (17) present convincing arguments in favor of the role of phytoalexins in disease resistance; however, it is not certain that phytoalexins constitute a mechanism of resistance in all plants. Antibiotic compounds synthesized in response to infections (phytoalexins) have been isolated from a number of diverse plants infected by various organisms. Structurally, phytoalexins range from

Received for publication 1 September 1981.

[1]Symposium presented at the Annual Meeting of the Society of Nematologists, 16-19 August, 1981, Seattle, Washington.

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relatively simple straight chain compounds to complex heterocyclic compounds. Grisebach and Ebel (10) chemically classified phytoalexins into isoflavanoids, sesquiterpenes, furanoterpenoids, polyacetylenes, dihydrophenanthrenes, and miscellaneous compounds. Bell (3) classified phytoalexins into stilbenes, coumarins, polyenes, flavanoids, isocoumarins, terpenoids and furanoacetylenes. Phytoalexins representative of various classes of compounds are shown (Fig. 1). The biosynthesis of phytoalexins have been reviewed (3,10); most are derived from acetate condensed with cinnamic acid (for flavanoid and stilbene type phytoalexins) or mevalonic acid (for terpenoid type phytoalexins) or fatty acid metabolism (for polyacetylene type phytoalexins).

Many organisms and a multitude of abiotic substance stimulate phytoalexin synthesis. Substances that stimulate synthesis are called "elicitors" (16). Abiotic elicitors such as heavy metal salts (4), ultraviolet radiation (6), and low temperatures (9) have been reported. Besides whole microorganisms, fungal cell walls and fungal products in culture filtrates (2,8) also elicit phytoalexin synthesis. These are considered biotic elicitors. The fact that the synthesis of many phytoalexins can be turned on by a number of different elicitors is often argued as demeaning their importance. In fact, nonspecific elicitation may be a virtue for general or broad range mechanisms of resistance. The mechanism by which elicitors stimulate phytoalexin synthesis is not known. Bell (3) concluded from the literature that all elicitors, at effective doses, adversely affect membrane permeability, and that phytoalexins are consistently associated with a necrogenic response in resistant hosts. From these observations Bell builds a hypothesis for a mechanism of action: he suggests that elicitors bind to cell walls in a manner similar to wall binding of phytotoxins. That is, elicitors attach to oligosaccharide binding lectins (such as galactan- and glucosamine-binding lectins) on host membranes or walls. The binding of the elicitor then impairs the permeability of the membrane (as does phytotoxin) which in turn leads to phytoalexin production and subsequent cell death. This hypothesis is attractive but whether it will withstand kinetic analysis remains to be determined.

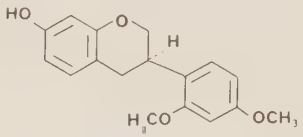
A number of host responses may occur upon elicitation. Differential accumulation of phytoalexins in susceptible and resistant hosts is often encountered and can form the basis of resistance. However, toxic concentrations of phytoalexins without the attendant resistant response may also be present. This absence of a resistant response in the presence of toxic concentrations of phytoalexin may be explained by the rate at which accumulation occurs (22) or by the sites at which phytoalexins accumulate. Additionally, oxidation of endogenous nontoxic derivatives to toxic phytoalexins during extraction and chemical work up may occur. If phytoalexin accumulation is not timely or if accumulation is not anatomically localized to contain the development or spread of the invading organism, toxic concentrations may accumulate but a susceptible host response will be observed. Both susceptible and resistant host cultivars will respond initially to elicitation, but the rate of accumulation is usually faster in resistant hosts. Additionally, phytoalexins may occasionally fail to be effective because some pathogens have a mechanism to metabolically detoxify phytoalexins (24). Although detoxification is not consistently associated with virulence (3), it can be considered a defense reaction of the pathogen to the host plant.

For phytoalexins to effect resistance, they must fulfill certain requirements of a time-space-effect (T-S-E) interrelationship (28). That is, they must be produced at the proper time (usually within 4–5 days after infection), localized in the proper cells or tissues (i.e., in close proximity to the pathogen), and have some type of antibiotic effect on the pathogen (induce death, inhibit development, or prevent spread). Histochemical or histological studies are often the best way of elucidating the T and S criteria of the relationship.

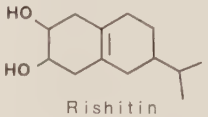
With this backround on phytoalexins, let me now turn to some specific examples of phytoalexins associated with plant resistance to nematodes.

SPECIFICS

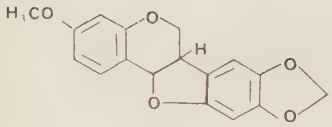
Considering the variety of pathogens



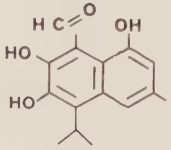
Sativan



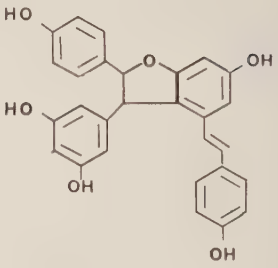
Rishitin



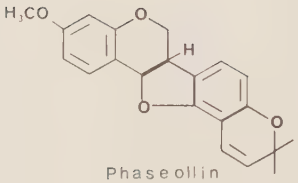
Pisatin



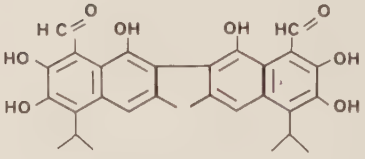
*Hemigossypol



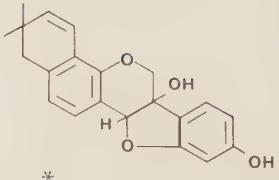
Viniferin



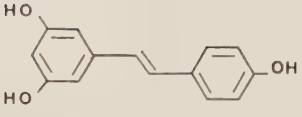
Phaseollin



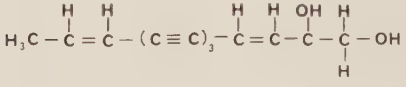
*Gossypol



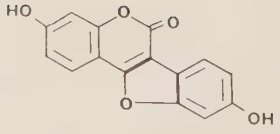
*Glyceollin



Resveratrol



Safynol



*Coumestrol

Fig. 1. Examples, from various classes of compounds, of phytoalexins, some of which (*) have been implicated in plant resistance to nematodes.

that elicit phytoalexin synthesis in plants, it is not surprising that nematodes can also induce such host responses. The first nematode-plant interaction study that specifically reported the induction of phytoalexin synthesis was that of Abawi et al. (1). They inoculated red kidney bean (*Phaseolus vulgaris*) with *Pratylenchus penetrans* and 5 days later extracted phaseolin (Fig. 1), a bean phytoalexin, from the nematode infected tissue. Since the beans were inoculated under sterile conditions with axenized nematodes and the noninoculated control plants did not produce detectable phaseolin, it is reasonable to infer that the infected plants produced phaseolin (59 μg/g root tissue) in response to infection by *P. penetrans*. Unfortunately, when *P. penetrans* larvae were exposed to 47 μg phaseolin/ml for 16 h they were not adversely affected. Thus, although phaseolin, a known phytoalexin, was synthesized by the host plant in response to infection by a nematode, it apparently did not constitute a resistance factor to *P. penetrans* in beans. Since phaseolin failed the effect aspect of T-S-E requirements, it is not necessary to consider the other two aspects. However, bean is known to produce several other phytoalexins, such as kievitone, and their antihelminthic activity should be investigated.

Phaseolus lunatus vs. Pratylenchus scribneri: The first specifically identified nematode-induced phytoalexin that appeared to be the active principle of a mechanism of resistance was identified in lima bean (*Phaseolus lunatus*). This plant produces phytoalexins in response to infection by various fungal and bacterial pathogens and is a poor host for *Pratylenchus scribneri;* hypersensitive lesions develop soon after infection by the nematodes. Rich et al. (21), therefore, hypothized that lima bean resistance to *P. scribneri* might be accounted for by nematode-induced synthesis of a phytoalexin.

To test their hypothesis, they isolated from nematode-infected lima bean roots four coumestans that accumulated concomitant with the hyper-sensitive (necrotic) response. One of the coumestans was identified as coumestrol (Fig. 1); a second was tentatively identified as psoralidin; the remaining two were not identified. By 1 day after inoculation, more than 40 μg coumestrol per g root tissue was extracted; by 4 days after inoculation, concentration exceeded 70 μg coumestrol/g tissue. Psoralidin accumulated more slowly but exceeded 40 μg/g tissue by 4 days after inoculation. During the same period, levels of coumestrol and psoralidin in healthy plants did not exceed 10 μg/g tissue. When lesions (necrotic tissue) were carefully dissected from adjacent nonnecrotic tissues, coumestrol and psoralidin levels in lesions were 89 and 39 μg/g, respectively. Neither coumestan exceeded 6 μg/g in adjacent nonnecrotic tissue. Thus, we see that phytoalexins accumulated at the sites of nematode attack.

To test the effect, coumestrol was bioassayed against the nematode species that induced its accumulation. Exposure of *P. scribneri* to coumestrol at 5 μg/ml significantly reduced motility compared to water-treated controls; the ED_{50} was 10–15 μg/ml. Exposure to 25 g coumestrol/ml for 48 h severely inhibited the motility of *P. scribneri,* but the effect was reversed when the nematode was removed from the phytoalexin. In similar bioassays, the phytoalexin had no adverse effect on *Meloidogyne javanica.*

In a complementary test, snap bean (*Phaseolus vulgaris*), which is a good host for *P. scribneri* and does not form a necrotic lesion in response to infection, was analyzed for phytoalexin production. Noninoculated root tissue accumulated coumestans in low levels comparable to noninoculated lima bean; this indicated an extant capacity to produce coumestans. However, additional accumulation in response to infection did not occur.

The fulfillment of the T-S-E requirements is difficult to assess for this plant-nematode interaction. The phytoalexin accumulates in substantial amounts within 4 days after inoculation and concomitant with the host hypersensitive response (time), it accumulates at the site of nematode attack (place), and it inhibits the motility of the nematode (effect). But, because of the vagrant nature of *P. scribneri,* the phytoalexin must quickly accumulate to sufficient levels to immobilize the nematode. It is conceivable that the nematode elicits phytoalexin

synthesis but that the phytoalexin accumulates to the observed levels after the nematode has migrated to nonelicited tissue. In which case it would be hard to imagine an effective mechanism of resistance.

Glycine max vs. Meloidogyne incognita. The most detailed and impressive study of a nematode-induced phytoalexin type mechanism of resistance is that reported by Kaplan et al. (13,14). These studies analyzed the responses of two soybean cultivars ('Centennial' and 'Pickett 71') to infection by two closely related nematodes (*M. incognita* and *M. javanica*). The host responses to these nematodes were as follows:

Host	*M. incognita*	*M. javanica*
Centennial	Resistant	Susceptible
Pickett 71	Susceptible	Susceptible

The phytoalexin hypothesis for this model requires, among other things, that phytoalexin accumulation occur with the host-nematode interaction that results in a resistant response but not with the susceptible responses.

Root extracts from the four host-nematode combinations were made at various times after inoculation and analyzed for the presence of glyceollin (Fig. 1), a soybean phytoalexin. Constitutive low concentrations (15 μg/g root) of glyceollin were detected in the healthy roots of both soybean cultivars. After inoculation, however, additional accumulation occurred only in Centennial roots inoculated with *M. incognita* (the resistant host-nematode interaction). By 3 days after inoculation nearly 40 μg glyceollin/g root tissue was detected, and by 7 days after inoculation glyceollin exceeded 70 μg/g.

To determine more precisely the sites of localization of glyceollin, the roots of 5-day-old infections were mechanically decorticated and the cortex and stele were analyzed separately. The stele of Centennial infected by *M. incognita* contained almost 130 μg glyceollin/g stele tissue; the concentration in the stele from the three susceptible interactions was about 50 μg/g stele tissue. Glyceollin in cortex was highest in the resistant host-nematode interaction, but it did not exceed 30 μg/g cortex; glyceollin concentrations in the cortex of the susceptible host-nematode interactions did not exceed 20 μg/g cortex. Thus, it appeared that the bulk of the glyceollin, especially that synthesized in the resistant host in response to infection, accumulated in the stele.

The efficacy of glyceollin on inhibiting the motility of *M. incognita* and *M. javanica* larvae was determined. Exposure to 60 g/ml for 24 h had no apparent effect on *M. javanica*. However, about 70% of the *M. incognita* larvae were adversely affected by 15 μg/ml, and the ED_{50} was determined to be 11 μg/ml. Because affected nematodes regained motility upon removal of glyceollin from the test medium, Kaplan et al. (13) concluded glyceollin was nematistatic, but not nematicidal, to *M. incognita*.

In additional studies, Kaplan et al. (14) partially defined the mechanism by which glyceollin might function as a nematistatic phytoalexin. The rate of oxygen consumption by *M. incognita* larvae was reduced 50% by 48 μg glyceollin/ml; at the ED_{50} concentration for inhibition of *M. incognita* motility (11 μg/ml), oxygen consumption was reduced about 13%. Glyceollin did not inhibit nematode choline esterase activity. In soybean mitochondria the electron transport system was inhibited by 1 μg glyceollin/ml but mitochondrial membranes were not adversely affected and oxidative phosphorylation was not uncoupled. These effects on soybean mitochondria were assumed to apply to nematode mitochondria (based on commonality of mitochondria from all organisms), and the data were interpreted to indicate that the primary action of glyceollin is the inhibition of the electron transport system. This interpretation does not explain why glyceollin does not affect *M. javanica* larvae; the commonality between *M. incognita* and *M. javanica* mitochondria must be closer than between *M. incognita* and soybean mitochondria. Kaplan et al. (14) suggested the possibility of differential uptake of glyceollin by the nematodes; one might also suggest differential degradation of glyceollin by the nematodes.

This system has a number of attributes in addition to fulfilling the T-S-E requirements. The fact that the nematode becomes sedentary soon after establishing a feeding

site increases the probability that it remains associated with the cells that accumulate the phytoalexin. It is also interesting that infection by *M. javanica* did not result in phytoalexin accumulation in the *M. incognita* resistant plants. Were phytoalexins not elicited, or were they metabolized by *M. javanica* as they were formed?

Gossypium hirsutum vs. M. incognita: A phytoalexin-type mechanism of resistance of cotton to the root-knot nematode has been reported by Veech and McClure (29) and Veech (26,27). The mechanism is predicated on the accumulation, in response to infection, of nematicidal concentrations of terpenoid aldehydes.

Cotton constitutively synthesizes gossypol and related terpenoids. The rate and amount of constitutive terpenoid accumulation is a function of the cultivar and has little or no relationship to the level of host resistance or susceptibility. Terpenoid aldehydes accumulate in the epidermis in all but 3–4 cm of the tips of noninfected cotton roots. No accumulation occurs in the stele, and only occasional cortical cells accumulate terpenoid aldehydes. Thus, constitutive accumulation does not occur in the portion of the root where the nematode penetrates, or in root cells near sedentary nematode feeding sites. Hence, it is of little consequence that concentrations of constitutive terpenoid aldehydes are not correlated to susceptibility or resistance, because the preinfectional terpenoid aldehydes are not anatomically localized to be effective against nematodes.

What is of consequence, however, is the accumulation of terpenoid aldehydes in host plants in response to infection. To demonstrate this putative mechanism of resistance, five cotton cultivars with different levels of resistance to *M. incognita* were selected for study; in order of decreasing resistance, the cultivars were 'Auburn 623,' 'N6072,' 'Clevewilt,' 'Deltapine 16,' and 'M-8.' The concentrations of five terpenoid aldehydes—hemigossypol, methoxyhemigossypol, gossypol, methoxygossypol, and dimethoxygossypol—were determined in the roots of each cultivar 5 days after inoculation with *M. incognita;* the concentrations in comparably aged noninoculated roots were also determined. The concentrations of each terpenoid aldehyde increased in response to infection, compared to the noninoculated controls, in the roots of the three most resistant cultivars, but it decreased in the two least resistant cultivars. Coefficients of correlation between infection-induced concentrations of methoxygossypol and the level of host resistance based on root-knot index, egg masses/g root tissue, and eggs/g root tissue, were .91, .96, and .97 ($P = .01$), respectively. This indicated that methoxygossypol accumulation in response to infection was directly proportional to the level of host resistance.

Because constitutive terpenoid aldehydes accumulated in roots at sites not likely to be effective against the sedentary nematode, new sites of accumulation had to be formed in response to infection. A histochemical study of infected roots demonstrated that within 4 days after inoculation, infection-induced terpenoid aldehydes accumulated in the resistant host in histochemically detectable amounts in the endodermis and stele at, or very near, the feeding site of the nematodes. Infection-induced terpenoid aldehyde accumulation was occasionally observed in the susceptible host, but it did not accumulate as rapidly as in the resistant host, nor did it encompass as many cells.

The effect of terpenoid aldehydes on the motility of *M. incognita* was determined by exposing larvae to a mixture of terpenoid aldehydes at various concentrations (based on gossypol equivalents) for various times. Exposure to 10 ppm for 24 h had little effect on larvae motility. Exposure to 50 ppm immotilized about 70% of the larvae, but the effect was reversed by a 24-h recovery period in the absence of terpenoid aldehydes. Exposure to 125 ppm for 5 h immotilized all the larvae, but 88% regained motility with a 24-h recovery period; only 17% regained motility after a 24-h exposure.

Considering the rate at which infection-induced terpenoid aldehydes accumulate in response to infection, and the highly localized nature of that accumulation, antibiotic levels of terpenoid aldehydes probably accumulate in the resistant plant concommitant with the nematode becoming sedentary.

CONCLUSIONS

The role of phytoalexins in the resistance of plants to nematodes has not been extensively explored, but the findings to date indicate that this is a fertile area of research. Fortunately, phytopathologists have already established much on which nematologists can capitalize. Nematologists interested in phytoalexin production need not proceed stochastically at isolating and identifying such antibiotic compounds. Although not absolute, a relationship seems to exist between the plant taxonomic family and the type of phytoalexins produced (11). The Leguminosae generally produce iso-flavanoid-type phytoalexins, Compositae usually produce polyacetylene phytoalexins. Malvaceae and Solanaceae generally produce terpenoid type phytoalexins. These generalities, together with the established literature on specific phytoalexins, constitute excellent starting points in searching for phytoalexins synthesized in response to nematode infection.

Other challenges in the area of phytoalexins and plant resistance to nematodes await our attention. Van Staden and Dimella (25) correlated constitutive cytokinin levels in the root to levels of susceptibility to *M. javanica,* and Bird and Loveys (5) reported increased cytokinin levels associated with nematode infection. Since Sziraki et al. (23) reported that exogenously applied cytokinin inhibits necrosis caused by mercuric chloride, and since mercuric chloride is a good elicitor of phytoalexins, one may speculate that cytokinins are responsible for the reduced or inhibited accumulation of phytoalexins in nematode susceptible plants. Or, if Bell's (3) hypothesis is correct and phytoalexin elicitors do bind to walls or membranes, we may speculate that the Concanavalin A binding sites on the head of *M. incognita* (18) represent a mechanism whereby some nematodes inhibit elicitation by binding the elicitors.

A slight delay in the elicitation of phytoalexins by sedentary endoparasites would seem to be ideal. Too rapid accumulation of phytoalexins could be energy inefficient, because the transitory nematode might detect sub-effective levels of the phytoalexin and migrate to nonelicited cells. New sites of infection-induced synthesis would then have to be established at additional energy expense to the plant. Ideally, phytoalexin accumulation should begin about the time the nematode becomes sedentary and progress fast enough to adversely effect its development.

I am reasonably convinced, and Kaplan and Keen (12) seem to concur, that phytoalexins can serve as effective mechanisms of resistance of plants to nematodes, expecially sedentary nematodes. We have only begun to catalog the phytoalexins that are synthesized in response to nematode infection and to understand how these compounds function in resistance. There is ample evidence to indicate that phytoalexin synthesis is ammenable to qualitative and quantitative genetic manipulation. Thus, the exploitation of phytoalexins can develop into a powerful tool for protecting plants from nematodes and thereby increase agricultural productivity.

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Potential of Tissue Culture for Breeding Root-knot Nematode Resistance into Vegetables[1]

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Abstract: Plant protoplast technology is being investigated as a means of transferring root-knot nematode resistance factors from *Solanum sisymbriifolium* into the susceptible *S. melongena*. *Solanum sisymbriifolium* plants regenerated from callus lost resistance to *Meloidogyne javanica* but retained resistance to *M. incognita*. Tomato plants cloned from leaf discs of the root-knot nematode resistant 'Patriot' were completely susceptible to *M. incognita*, while sections of stems and leaves rooted in sand in the absence of growth hormones retained resistance. Changes in resistance persisted for three generations. It is postulated that the exogenous hormonal constituents of the culture medium are modifying the expression of genetic resistance. *Key words:* review, protoplast, callus, *Solanum sisymbriifolium*, *Solanum melongena*, eggplant, tomato, *Meloidogyne incognita*, *Meloidogyne javanica*, root-knot nematodes, somatic hybridization.

Journal of Nematology 14(1):10-14. 1982.

It is generally accepted that polygenic horizontal resistance to nematodes would be more stable and effective for longer periods of time than vertical resistance conditioned by a single gene. This, in turn, is dependent upon the availability of sexually compatible and genetically diverse germ plasm sources. The gene pool in some crops for root-knot nematode resistance is large, and resistant plants can be readily identified for use in a plant breeding program. In others, the variability is either extremely narrow or nonexistent, necessitating the search for resistance in related species, which most often are incompatible with the cultivated species (7).

The eggplant (*Solanum melongena*), for example, is a vegetable crop in which no resistance to root-knot nematodes has been found (4). A potential source of resistance occurs in a related wild species, *S. sisymbriifolium*, (10). However, the two species are sexually incompatible. In order to transfer the resistance factors of the wild species into the eggplant, methods other than conventional breeding techniques would have to be utilized.

Recent advances in plant protoplast technology provide a mechanism for overcoming interspecific and intergeneric barriers to hybridization and for introducing new genetic information into plant cells without sexual reproduction. Production of a hybrid plant through protoplast fusion of vegetative cells is commonly referred to as somatic hybridization (2).

Recent reports on the hybridization of the potato (*Solanum tuberosum*) with the tomato (*Lycopersicon esculentum*) and that of *Arabidopsis* and *Brassica* by protoplast fusion have demonstrated the feasibility of this technology for hybridizing sexually noncompatible plant species (12,14).

In somatic hybridization, plant tissues from two parental sources are digested with a mixture of pectinase and cellulase in an osmotically stable solution to produce protoplasts. Protoplasts are naked cells surrounded by a plasm membrane which have the capacity to fuse spontaneously or to be induced to fuse by mixing them together in the presence of a high molecular weight polyethylene glycol (PEG). A small percentage of the protoplasts will fuse to form heterokaryons containing a mixed cytoplasm with two nuclei. The fused protoplasts are capable of cell wall regeneration, growth, and division. After cell division a single nucleus may be formed containing the chromosomes of each parent cell or, more frequently, selective elimination of chromosomes of one of the parental species takes place during cell division and tissue formation (18).

The formation of a true interspecific hybrid cell can be identified readily by fusing green mesophyll protoplasts of one plant partner with the light yellow to colorless protoplasts of another partner prepared from cell suspension cultures. The contrasting colors of the heterokaryons become ob-

Received for publication 1 September 1981.

[1]Symposium presented at the Annual Meeting of the Society of Nematologists, 16-19 August 1981, Seattle Washington. Cooperative investigations, Agricultural Research Service, U. S. Department of Agriculture and South Carolina Agricultural Experiment Station.

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scure after several division and it is next to impossible to identify the hybrid cells from the more numerous parental cells. It is possible, however, to select cell hybrids in a nutrient medium which favors the growth of the hybrid products or preferentially allows the growth of only the hybrid cells (18). The colony of cells is subcultured on a culture medium favoring callus formation. The callus is subsequently transferred to a medium containing an auxin and cytokinin to induce organogenesis.

This technique is being used as a means of transferring root-knot nematode resistance factors from *Solanum sisymbriifolium* into the eggplant, *S. melongena.*

This report presents results of our research leading to the somatic hybridization of the two plant species, but more importantly it presents data on changes in expression of root-knot nematode resistance in cloned plants.

ORGANOGENESIS

Protoplast isolation and culture: Viable clear protoplasts from cell suspension cultures of *S. sisymbriifolum* and green protoplasts from young leaves of eggplant cv. Florida Market were prepared by enzymatic digestion. By manipulating the culture medium with various auxin/cytokinin ratios, osmoticum, light, and temperature, protoplasts from each plant species were induced to form new cell walls, divide repeatedly, produce colonies of cells, and regenerate into whole plants (1).

Heterokaryons: Clear *S. sisymbriifolium* protoplasts were fused with green mesophyll eggplant protoplasts with PEG. The heterokaryons, however, did not divide beyond a few divisions and eventually died. The conditions necessary for sustaining viability and cell divisions of the hybrid cells are being investigated.

Regeneration of plant species from callus: In the early stages of this research callus cultures were established from stem pith parenchyma of *S. sisymbriifolium* and *S. melongena* on Linsmaier and Skoog basal salt medium supplemented with sucrose, indole-3-acetic acid (IAA), (γ,γ-dimethyl-allyl-amino)–purine (2iP) and 2,4-dichlorophenoxyacetic acid (2,4-D). Plants were regenerated from *S. sisymbriifolium* callus on a basal salt medium containing a combination of high concentrations (74.9 μM) of the cytokinin, 2iP, and low concentrations (17.1 μM) of the auxin, IAA (5). Regeneration of *S. melongena* callus required a more complex protocol (11).

Characteristics of plants regenerated from callus: Morphological and cytological aspects of plants regenerated from callus of both *Solanum* species differed from original seed plants. Leaves of regenerated *S. sisymbriifolium* and *S. melongena* were thicker and less deeply lobed and the stomates were larger and contained more chloroplasts than seed plants. Cytological examination of pollen microspores of both species showed 24 chromosomes (n = 12), indicating that they were tetraploids (6,11).

REGENERATED PLANT RESPONSE

Since the plant morphology and cytology varied after regeneration, the regenerated plants were tested for resistance to root-knot nematodes after being selfed for two generations.

The usual response of eggplant roots to infection by *M. incognita* or *M. javanica* is the characteristic galling. The vascular cylinder is disrupted by the development of 6–8 large multinucleate, thick-walled giant cells which surround the nematode's head. Females assume a saccate shape in about 25 days and oviposit a few days later.

Solanum sisymbriifolium is also readily invaded by root-knot larvae. However, instead of galls, roots form swellings, primarily by a hypertrophy of the cortical parenchyma cells. At the nematode feeding site, tracheid continuity is disrupted by the development of small, thin-walled giant cells. Most larvae did not develop beyond the late second stage. The giant cells are not adequate to provide sufficient nutrition for nematode development.

Regenerated plants of both species exhibited the typical phenotypic root response described above after penetration by either *M. incognita* or *M. javanica* larvae. Progeny (S_1 and S_2) of regenerated *S. sisymbriifolium* plants retained resistance to *M. incognita.* Although root swellings were observed, no eggs were recovered. However, regenerated plants were more susceptible than seed plants to *M. javanica.* Larvae developed

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RESEARCH NOTES

Erratum: Pages in JOURNAL OF NEMATOLOGY 14(3) should have been numbered 279-426.

Information for Contributors

Original papers on basic, applied, descriptive, or experimental nematology are considered for publication. Other categories include research notes (not longer than four pages of copy, including tables and figures); reviews developing new concepts, hypotheses, or theories; and abstracts of papers presented at the annual meetings. At least one author must be a member of the Society of Nematologists, except that nonmember staff of subscribing institutions outside the United States or Canada may submit one paper per volume.

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Editorial page change: In order to partially cover the cost of publishing manuscripts in this journal, the Society suggests a contribution of $35 per printed page, as it is impossible to meet the demand for journal space without some financial assistance. Payment is not a condition of publication, however, since articles are accepted on the basis of merit.

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